REMARKS

Claims 1, 25, 29-36, 40-46, 49-55, 60-66, 68 and 70-77 are currently pending in the application. Claims 29-31 and 49-51 are currently amended. Upon entry of the present amendments, claims 1, 25, 29-36, 40-46, 49-55, 60-66, 68 and 70-77 will be pending in this application. Claim 26 is amended to depend from claim 25 rather than claim 1. Support for the amendment to claims 29-31 and 49-51 is found at page 5, last paragraph.

It is submitted that no new matter has been introduced by the present amendments, and entry of the same is respectfully requested. By these amendments, Applicant does not acquiesce to the propriety of any of the Examiner's rejections and does not disclaim any subject matter to which Applicant is entitled. *Cf. Warner Jenkinson Co. v. Hilton-Davis Chem. Co.*, 41 U.S.P.Q.2d 1865 (1997).

The Written Description Rejection Under 35 U.S.C. § 112, First Paragraph Should Be Withdrawn

Claims 29-31 are rejected under 35 U.S.C. § 112, first paragraph, with the Patent Office contending that the specification does not describe the subject matter now claimed. Office Action at pages 2-4.

Applicant asserts that this rejection is now moot and respectfully requests that the rejection be withdrawn. Specifically, claims 29-31 are rejected because the Patent Office objects to the presence of the term "culturing," and, with respect to claim 31, the phrase "at least twenty-four hours." Without acquiescing to the propriety of the rejection, and solely to expedite allowance, Claims 29-31, have been amended to no longer recite these terms. Support for the claim amendments can be found in the specification at page 5, last paragraph.

The Enablement Rejection Under 35 U.S.C. § 112, First Paragraph, Should Be Withdrawn

The Patent Office maintains the rejection of claims 1, 25, 29-36, 40-46, 49-55, 60-66 and 68, and newly rejects claims 70-77, under 35 U.S.C. § 112, first paragraph, as allegedly non-enabled. Office Action at pages 4-9. Applicant traverses as follows.

Analysis of enablement requires a determination of whether the "disclosure, when filed, contained sufficient information regarding the subject matter of the claims as to enable one skilled in the pertinent art to make and use the claimed invention." MPEP 2164.01 at page 2100-178. One skilled in the art is presumed to use the information available to him in attempting to make or use the claimed invention. *See Northern Telecom, Inc. v. Datapoint Corp.*, 908 F.2d 931, 941 (Fed. Cir. 1990). The standard for determining whether a claim is

enabled or not is whether it requires undue experimentation to practice. *Id.*; *see also Mineral Separation v. Hyde*, 242 U.S. 261, 270 (1916); *In re Wands*, 858 F.2d 731, 737, 8 U.S.P.Q.2d 1400, 1404 (Fed. Cir. 1988). The mere fact that something has not previously been done is not, in itself, a sufficient basis for rejecting all applications purporting to disclose how to do it. *Gould v. Quigg*, 822 F.2d 1074, 1078 (Fed. Cir. 1987).

The basis for the rejection appears to be the Patent Office's contention that the specification does not teach whether the cells collected via the claimed methods are indeed stem cells. Office Action at page 5. Specifically, the Patent Office contends that the specification fails to disclose the cells collected by the claimed methods necessarily contain stem cells. Office Action at page 9. In addition, the Patent Office requests that the Applicant "provide evidence that the isolated cells from perfusion solution are indeed stem cells, and $CD34^+$ stem cells." Office Action at pages 8-9.

In response, Applicant asserts that the teachings provided in the application as originally filed, coupled with the state of the art at the time the application was first filed, allows one of skill in the art to practice the full scope of the invention without having to resort to undue experimentation. In particular, the teachings provided in the specification allow one of skill in the art to routinely practice the claimed methods of collecting stem cells via perfusion of exsanguinated mammalian placentas which do, indeed, result in the presence of stem cells, including $CD34^+$ stem cells, in the perfusion solution used to perfuse the exsanguinated placenta.

At the outset, Applicant respectfully points out that teachings presented throughout the application that the methods now claimed result in the collection of stem cells. In addition, Applicant points out that the application discloses working examples of the claimed methods that successfully result in the collection of cells that include stem cells, including $CD34^+$ stem cells. *See* specification pp. 8-12. The instant specification also provides a characterization of cells collected by the methods. *See* specification pp. 11-12.

As corroboration that the teaching provided in the specification, coupled with the state of the art at the time the application was first filed, does indeed, allow one of skill in the art to routinely collect stem cells via perfusion of exsanguinated mammalian placentas, Applicant submits herewith the Declaration of Qian Ye, Ph.D. under 37 C.F.R. § 1.132 (the "Ye Declaration"), which is attached hereto as Exhibit 1.

Briefly, the experiments summarized in the Ye Declaration show, via functional and biomarker assays, that the cells in the perfusate solution resulting from the claimed methods include stem cells, including $CD34^+$ stem cells. Specifically, the first set of experiements

described in the Ye Declaration (Ye Declaration, paragraphs 8-10; Ye Declaration Exhibit B) relate to experiments performed according to the teachings presented in the instant application (Ye Declaration, paragraph 8) that utilize fluorescence activated cell sorting analysis (FACS) to show that cells collected from placenta perfusate obtained according to the teachings in the specification contain stem cells, including $CD34^+$ stem cells. (Ye Declaration, paragraph 9). These experiments also describe colony forming unit (CFU) functional assays demonstrating that the placenta perfusate not only contains stem cells, but contains functional stem cells. (Ye Declaration, paragraph 10).

The second set of experiments described in the Ye Declaration (Ye Declaration, paragraphs 11-15; Ye Declaration Exhibit C) also relate to experiments performed according to the teachings provided in the instant Application. (Ye Declaration, paragraph 11). The results of these experiments demonstrate that cells contained in placenta perfusate obtained according to the teachings provided in the specification include stem cells, and, in fact includes a higher proportion and total number of primitive stem cells ($CD34^+$ $CD38^-$; corresponding to a population of cells that contain the long-term marrow repopulating activity) than that of cord blood. (Ye Declaration, paragraph 13).

These experiments also demonstrate that the stem cells present in the placenta perfusate include both hematopoietic and non-hematopoietic stem cells. In particular, the results of the CFU analyses show the cells in the placenta perfusate include hematopoietic stem cells that give rise to erythrocytes, cells that give rise to granulocyte and macrophage cells, and cells that represent more primitive hematopoietic stem cells that can give rise to granulocytes, erythrocytes, monocytes, and megakaryocytes. (Ye Declaration, paragraph 14). Moreover, the experiments show that the cells in the placenta perfusate include $SH2^+$ $CD45^-$ and $SH3^+$ $CD45^-$ cells, which are indicative of non-hematopoietic stem cells.

Because, as set forth herein, the specification does, indeed, enable the claimed methods for the collection of stem cells, Applicant respectfully requests that the Examiner withdraw the rejection of claims 1, 25, 29-36, 40-46, 49-55, 60-66, 68 and 70-77 under 35 U.S.C. § 112, first paragraph.

The Rejection under 35 U.S.C. § 112, Second Paragraph, Should be Withdrawn

The Examiner rejects claims 49-51 under 35 U.S.C. § 112, second paragraph, as allegedly indefinite. Office Action at page 6. Applicant traverses as follows.

Without acquiescing to the propriety of the rejection, and solely to expedite allowance, Applicant has amended claims 49-51 to clarify that which has always been considered the claimed invention. With these amendments, Applicant asserts that the

rejection is now moot and respectfully request that the rejection under 35 U.S.C. § 112, second paragraph, be withdrawn.

CONCLUSION

Applicant respectfully requests that the above remarks and accompanying documents be entered in the present application file. An early allowance of the present application is respectfully requested.

No fee is believed due for this Amendment. However, if a fee is due, please charge such fee to Jones Day Deposit Account No. 50-3013 (order no. 501872-999099).

Respectfully submitted,

Date: April 15, 2005

39,201

Nikolaos C. George (Reg. No.)
JONES DAY
222 East 41st Street
New York, NY 10017
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of:	Robert J. Hariri	Confirmation No.:	7788
Serial No.:	10/004,942	Art Unit:	1632
Filed:	December 5, 2001	Examiner:	Li, Qian Janice
For:	METHOD OF COLLECTING PLACENTAL STEM CELLS	Attorney Docket No::	9516-100-999

DECLARATION OF QIAN YE, Ph.D. UNDER 37 C.F.R. § 1.132

I, Qian Ye, Ph.D., declare the following:

1. I have been employed for nearly four years by Anthrogenesis Corporation (now part of Celgene Corporation) as a Senior Scientist. It is my understanding that Anthrogenesis Corporation is the assignee of record the above-captioned patent application.

2. I earned a Bachelor of Science degree in Microbiology at Xiamen University (China) in 1986. In 1990, I earned a Master of Science degree in Botany, also at Xiamen University (China). I earned a Doctor of Philosophy degree in Biomedical Science at the Mount Sinai Medical Center, The City University of New York in 1996. In addition, I have received postdoctoral training at Colombia University (1996-1997) and Memorial Sloan Kettering-Cancer Center (1997-2001). I have attached a copy of my Curriculum Vitae ("CV") to this Declaration as Exhibit A.

3. I have extensive experience in the area of stem cell technology. As the attached CV indicates, my expertise includes extensive experience in the design, execution and analysis of experiments relating to the production and proliferation of stem cells, as well as in the evaluation of such stem cells. I have published no fewer than five (5) abstracts related to stem cells. Specifically, for example, I have presented abstracts related to the expansion and differentiation of $CD34^+$ stem cells, the differentiation of stem cells from

the placenta into neural cells, and the recovery of stem cells from a cultivated, postpartum human placenta.

4. In addition, in my capacity as Senior Scientist at Anthrogenesis Corporation, I have executed and/or supervised experiments related to stem cells. Specifically, for example, I have designed and implemented research programs on the isolation, establishment, and the differentiation of stem cells from the human placenta and from cord blood.

5. I have read and I am familiar with the above-captioned patent application, Serial No. 10/004,942 ("the '942 application"), filed December 5, 2001, and the claims I understand are currently pending in the '942 application. It is my understanding that an Office Action, mailed December 16, 2004, is outstanding in connection with the '942 application. I have also read and am familiar with the claims that will be pending in the '942 application upon entry of the response to the outstanding office action that is being filed concurrently herewith in the United States Patent and Trademark Office.

6. As described in the following paragraphs, experiments performed by me, or familiar to me, demonstrate that the teaching provided in the specification of the '942 application, coupled with the state of the art at the time the '942 application was first filed, allows for routine practice of the methods being claimed in the '942 application such that stem cells, including $CD34^+$ stem cells, can routinely be collected from exsanguinated mammalian placentas.

7. In particular, the results presented in Exhibits B and C, attached hereto, demonstrate that a detectable amount of stem cells can be collected from exsanguinated mammalian placentas that have been drained of cord blood, flushed of residual blood, and then perfused.

8. The experiments presented in Exhibit B were performed according to the teaching presented in the '942 application. *See*, for example, p. 4, l. 1, to p. 5, l. 26, and pp. 8-10 describing placental processing, conditioning, and perfusion methods.

9. As set forth in Exhibit B, FACS (fluorescence activated cell sorting) analyses demonstrate that cells collected from placenta perfusate obtained according to the teaching presented in the '942 application contain stem cells, including $CD34^+$ stem cells.

10. As also set forth in Exhibit B, colony forming unit (CFU) assays demonstrate that the cells collected from placenta perfusate obtained according to the teaching presented in the '942 application include not only stem cells, but functional stem cells.

11. The experiments summarized in Exhibit C, attached hereto, were performed according to the teaching provided in the '942 application. *See*, for example, p. 4, l. 1, to p. 5, l. 26, and pp. 8-10, describing placental processing, conditioning, and perfusion methods, and p. 6, ll. 3-6, and pp. 10-11, describing isolation of cells. The experiments summarized in Exhibit C are described in more detail in an abstract, Wang *et al.*, 2001, Blood **98**:183a, which is attached to Exhibit C as Exhibit C-1, and in a conference poster session presented on December 8, 2001 (*see* Exhibit C-2, attached to Exhibit C).

12. First, as set forth in Figure 1 (Exhibit C-1), mononucleated cells (MNCs) were harvested from placentas maintained for either 2 to 12 hours or for 12 to 24 hours. More MNCs were harvested from the placentas that were maintained for 12 to 24 hours than from the placentas maintained for 2 to 12 hours.

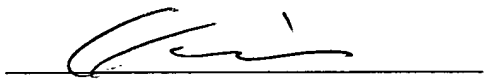
13. Figure 2 (Exhibit C-2) provides the results of FACS analyses of $CD34^+CD38^-$ cells in placenta perfusate compared to cord blood. Table 1 (Exhibit C-2) shows quantitation of the $CD34^+CD38^-$ cells in the placenta perfusate compared to cord blood units. These data demonstrate that not only does placenta perfusate contains stem cells, but it contains a higher proportion and total number of primitive stem cells ($CD34^+CD38^-$ cells) than that of cord blood. It is thought that the $CD34^+CD38^-$ cells contain the long-term marrow repopulating activity.

14. Figure 3 (Exhibit C-2, attached hereto) shows three examples of CFU analyses of placenta perfusate MNCs. The placenta perfusates exhibited: CFU-E/BFU-E cells (66+/-

11% of the total CFU population), which represent stem cells that give rise to erythrocytes; CFU-GM cells (28+/-10% of the total CFU population), which give rise to granulocyte and macrophage cells, and CFU-GEMM cells (6+/-3% of the total CFU population), which represent more primitive hematopoietic stem cells that can give rise to granulocytes, erythrocytes, monocytes, and megakaryocytes.

15. The studies also demonstrate that there are $SH2^{+}CD45^{-}$ and $SH3^{+}CD45^{-}$ type stem cells in the placenta perfusate. (*See* Exhibit C-1; Figure 4, Exhibit C-2.) Cells of these biomarker designations are indicative of non-hematopoietic stem cells. The data also reveal a higher amount of these cells in placenta perfusate compared to that from cord blood.

16. All statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that the statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and such willful false statements may jeopardize the validity of the application or any patents issuing thereon.



April, 13 2005

Qian Ye, Ph.D. Date

Qian Ye Ph.D.

Date of Birth: 6-8-1965
SSN: 119-78-9568
Nationality: US citizen

EDUCATION:

1991-1996	Ph.D., Biomedical Science, Mount Sinai Medical Center, The City University of New York
1986-1990	M.S., Major in Botany, Xiamen University, China
1982-1986	B.S., Major in Microbiology, Xiamen University, China

POSTDOCTORAL TRAINING:

1997-2001	Cell Biology Program, Memorial Sloan Kettering-Cancer Center
1996-1997	Departments of Medicine and Pathology Columbia University College of Physicians and Surgeons

EXPERTISE:

Developmental Hematopoiesis

-Extensive experience in experimental design, data collection and analysis of proliferation and differentiation of hematopoietic stem cells from human and mouse using in vitro and in vivo systems

-Assays in evaluating hematopoietic stem cells including isolation of hematopoietic stem cells, colony forming cell assays, cobble-stone area assays (CAFCs), long-term bone marrow culture, long-term initiating cell assays (LTCICs), CFU-S

Animal Experiences

-Use of retrovirus system to express target genes in hematopoietic stem cells and analysis their effects on hematopoiesis in mouse by xenoengraftment

- Use of mouse model to study hematopoiesis and immunology

Flow-Cytometry

-Hands on experience of Becton-Dickson to perform cell cycle analysis, apoptosis, two-color and multiple-color analysis on protein expression

Molecular Biology and Biochemistry

-Extensive experiences in cDNA, genomic library screening

-Gene function analysis using retrovirus expression system

-Yeast two-hybrid system in analysis of protein-protein interactions

-Major DNA techniques including recombinant DNA cloning, Southern blot, Northern blot, PCR, RT-PCR, luciferase system in analysis of transcription regulation

-Major protein techniques including expression and purification protein from bacteria and mammalian cells (small or large scale), SDS-PAGE, Western-blot chromatography, immunoprecipitation, ELISA

Cell Biology

-Mammalian cell culture including cell lines, primary cells and ES cells, establishment of cell lines, cellular fractionation, conventional and confocal immunofluorescence microscope

AWARDS AND HONORS:

1999-2001	PHS Fellowship Award, National Heart, Lung, and Blood Institute, NIH
1996-1997	American Liver Foundation Postdoctoral Research Fellowship
1995-1996	American Liver Foundation Student Research Award
1995	American Society for Cell Biology Annual Meeting Travel Award
1994-1995	American Liver Foundation Student Research Award

RESEARCH AND WORKING EXPERICENCE:

Senior Scientist (5/2001-present)

Celgene, Cellular Therapeutics Division (Anthrogenesis Corp)

Responsibilities:

a. Design and implement research programs on the isolation, establishment, and multiple lineage differentiation stem cells from human placental and cord blood
b. Research programs for cell therapy pre-clinical studies
c. Assay development to study effects of small molecule drugs on the proliferation and cytokine directed differentiation of hematopoietic stem cells.
d. Assays development to study the effects of small molecules drugs on angiogenesis

Research Fellow (5/1997-5/2001)

Laboratory of Developmental Hematopoiesis, Memorial Sloan-Kettering Institute, New York
Mentor: Dr. Malcolm A.S. Moore

Project: Notch signaling on the regulation of hematopoietic stem cells

Research activities:

a. Cloning of cDNA for human Notch ligand Delta-like-1 (hDll1)
b. Expression and purification of Notch binding domain, the (DSL) domain, of hDll1 as a recombinant protein and studied its roles in regulating the proliferation and differentiation of hematopoietic stem cells.
c. Using retroviral vectors to express hDll1 and Notch receptors in mouse/human hematopoiteic stem/progenitor cells to analyze their roles in regulating hematopoiesis in vitro and in vivo using NOD/SCID mouse

Postdoctoral Fellow (4/1996-5/1997)

Department of Medicine and Cell Biology, Columbia University, New York
Mentor: Howard J. Worman, M.D.

Research Activities:

Functional analysis of domain-specific interactions between heterochromatin protein family and inner nuclear inner membrane protein lamin B receptor (LBR)

Graduate Research Assistant (9/1991-4/1996)

Department of Molecular Biology, Mount Sinai Medical Center, New York.
Mentor: Howard J. Worman, M.D.

Ph.D thesis: Protein-protein and protein-chromatin interactions at the nuclear envelope.

a. cDNA library screening, cloning of cDNA for human LBR, a nuclear membrane protein
b. Analysis of the interaction between LBR with DNA, chromatin and nuclear lamins
c. Analysis of interactions between major lamin proteins by yeast two-hybrid system
d. Identification of interactions between LBR and HP1 proteins with yeast two-hybrid screening

PUBLICATIONS:

1. **Ye, Q.,** Shieh, J-H, Morrone, G. and Moore, M.A.S. (2004) Expression of constitutively active Notch4 (Int-3) modulate myeloid proliferation and differentiation and promote expansion of hematopoietic progenitors. Leukemia. 2004 18:777-87.
2. Han, W., **Ye, Q.**, and Moore, MAS. (2000) A soluble form of human Delta-like-1 inhibits differentiation of hematopoietic progenitor cells. Blood 95:1616-1625.
3. **Ye, Q.**, Callebaut, I., Pezhman, A., Courvalin, J.C. and Worman, HJ. (1997). Domain-specific interactions of human HP1-type chromatin proteins and inner nuclear membrane protein LBR. J. Biol. Chem. 272:14983-14989.
4. **Ye, Q.**, and Worman, HJ. (1996). Interaction between an integral protein of the nuclear envelope inner membrane and human chromodomain proteins homologous to Drosophila HP1. J. Biol. Chem. 271:14653-14656.
5. Lin, F., Noyer, CM, **Ye, Q.**, Courvalin, J.C., and Worman, HJ. (1996). Autoantibodies from patients with primary biliary cirrhosis recognize a region within the nucleoplasmic domain of inner nuclear membrane protein LBR. Hepatology. 23:57-61.
6. **Ye, Q.**, and Worman, HJ. Protein-protein interactions between human nuclear lamins expressed in yeast. (1995) Exp. Cell Res. 219:292-298.
7. **Ye, Q.** and Worman. HJ. (1994). Primary structure analysis and lamin B and DNA binding of human LBR, an integral protein of the nuclear envelope inner membrane. J. Biol. Chem. 269:11306-11311.

BOOK CHAPTERS:

1.Moore, MAS, Han, W., and **Ye, Q.**, (2000). Notch signaling during hematopoietic development. In "Developmental Biology of Hematopoiesis" ed. Zon LI. Oxford Univ. Press

2. **Ye, Q.**, Barton, RM., and Worman, HJ. (1998). Nuclear Lamin-binding proteins. Subcell Biochem 31:587-610.

SELECTED ABSTRACTS:

1. **Ye, Q.,** Payvandi, F., Wu, L., Zhang, L., Muller, G., Chen, R., McClellan, S., Wang, J., Khorshidi, M., Magidson, J., Stengel, J., Stirling, D., and Hariri. R. (2002). *Novel IMID Drugs enhance expansion and regulate differentiation of human cord blood CD34+ cells with cytokines*. The American Society of Hematology 44th Annual Meeting. Philadelphia, PA. Dec.6-10. Blood. (2002) 100: Abstract No.4099
2. Wang, J., Struck, M., A., McClellan, S., **Ye, Q.**, Maclsaac, S., Lakato, T., Stengel, J., and Hariri, R. (2002) 2nd Annual Conference on Mesenchymal and nonhematopoietic stem cells. Sept. 26-28. New Orleans. LO. *Stem cells from placenta differentiate into neural cells in vitro*. Abstract No.49.
3. Wang, Y, **Ye, Q**, Cioffi J, Khorshidi, M., Magidson, J., Katz, R., Maclsaac, S., and Hariri, R.. *Enhanced recovery of hematopoietic progenitor and stem cells from cultivated, postpartum human placenta*. The American Society of Hematology 43rd Annual Meeting, Orlando, Florida, Dec 7-11, 2001. Blood (2001) 98: Abstract No.769
4. **Ye, Q.**, Wang, Y., Cioffi, J., Khorshidi, M., Magidson, J., Katz, R., Maclsaac, S., and Hariri, R. *Recovery of Placental-Derived Adherent Cells with Mesenchymal Stem Cell Characteristics*. Blood (2001) 98: The American Society of Hematology 43rd Annual Meeting Orlando, Florida, Dec 7-11. Abstract No. 4260
5. **Ye, Q.**, and Malcolm A.S. Moore (2000) *An activated Notch 4 (Int-3) affects the proliferation and differentiation of hematopoietic stem/progenitor cells*. The American Society of Hematology 42nd Annual Meeting, San Francisco, California, Dec.1-5. Abstract No.280

Isolation of total nucleated cells containing stem cells from placenta perfusate from "ex-sanguinated" placentas.

Material and Methods

Human placentas were drained of cord blood in the hospital delivery room by standard cord blood collection methods. These placentas were then procured under full-informed consent of the donor and incubated at room temperature or at 5°C. All placentas in this study were processed and analyzed more than 2 hours after expulsion, but within less than 24 hours after expulsion.

The placentas were perfused with an isotonic solution (0.9% NaCl, PBS, or DMEM) through umbilical artery and veins. The placenta was first flushed with 250 mL of the perfusate, which was not included in the following Fraction-A and Fraction-B. The placenta was then perfused twice with 250 mL of the perfusion solution for each perfusion and the placental perfusate was collected as Fraction-A and Fraction-B (each with approximately 250 mL in volume) in a sterile pan.

The total cell number in each fraction was determined by using an automated cell counter. The hematopoietic stem cell (HSC) content of each fraction (Fraction-A and Fraction-B) was measured by flow-cytometry staining with anti-CD34 antibodies. The stem cell contents were also evaluated by culturing the cells in Metho-Cult (Stem Cell Technologies, Vancouver, Canada) to measure the colony-forming-unit (CFU) content of each fraction as a measure of functionality of the cells.

Results:

1. **Total nucleated cells (TNC) in placenta perfusate**

Figure 1 shows average TNC count results from 32 placentas studied. The mean TNC count was 611±472 million in Fraction-A and 489±299 million in Fraction-B. The total mean TNC count from both Fractions was 1100±771 million.

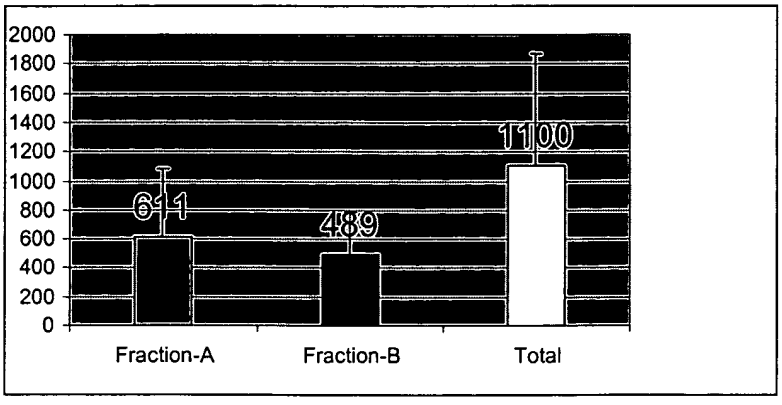


Figure 1: Total nucleated cells (TNC) collected from ex-sanguinated human placentas. Data shown are averaged from 32 placentas. Error bars indicate standard deviation (SD).

2. $CD34^+$ cells in the placenta perfusate

$CD34^+$ cells represent hematopoietic stem cells. The contents of $CD34^+$ cells were determined by FACS analysis in placental perfusate Fraction-A and Fraction-B. Figure 2 shows the frequency of $CD34^+$ cells as 0.17±0.08 (%) and 0.23±0.1 (%) in Fraction-A and Fraction-B, respectively. Thus, the results of this analysis demonstrate that stem cells are obtained from placentas that have been drained of cord blood and flushed to remove residual blood.

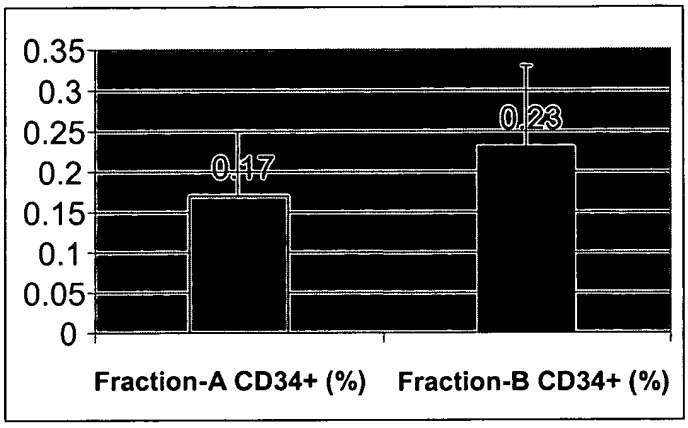


Figure 2: Frequency of CD34+ cells collected from exsanguinated human placentas. Data shown are averaged from 32 placentas. Error bar indicates standard deviation(SD).

3. Colony Forming Units (CFU) in the placenta perfusate

The CFU assay is a functional test for stem cells. Each CFU (colony forming unit) indicates a stem cell or progenitor cell. Fraction-A and Fraction-B TNC were plated in CFU culture medium (Stem Cell Technologies, Vancouver, Canada) and CFU numbers were measured 14 days later according to established protocols. Figure-3 shows CFU contents in placenta perfusate Fraction-A and Fraction-B and in the total combined perfusate. These data demonstrate the presence of functional hematopoietic stem cells in the placenta perfusate obtained from placentas that have been drained of cord blood and flushed to remove residual cord blood.

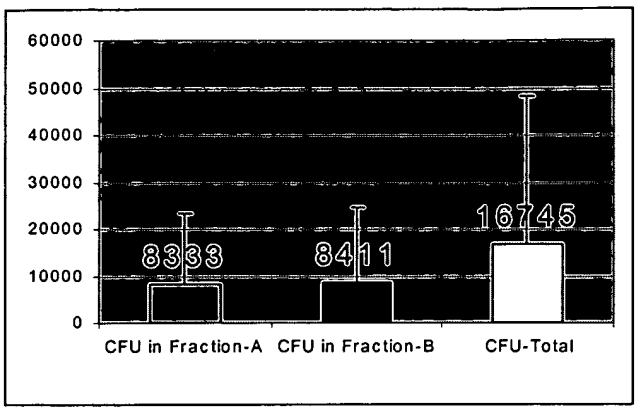


Figure 3. Colony-Forming Units in placenta perfusate from exsanguinated human placentas. Data shown are averaged from 29 placentas. Error bar indicates standard deviation (SD).

Characterization of stem cells in placenta perfusate from ex-sanguinated placenta.

The results of this study were presented on December 8, 2001 as part of a conference poster session (see Exhibit C-2, attached hereto) and in an abstract summarizing the data was published in Blood (November 16, 2001) 98:183a (see Exhibit C-1, attached hereto). In particular, the results present a detailed characterization of the stem cells in placenta perfusate obtained following the teaching provided in U.S. Patent Application Serial No. 10/004,942.

The placenta perfusates were obtained as described in the attached abstract and poster session materials. The placenta perfusate was purified by Ficoll-gradient centrifugation to generate "mononucleated cells" (MNCs).

The purified cell fractions were assayed for their TNC (total nucleated cells), $CD34^+CD38^-$ cells (more primitive stem cells) content and more detailed CFU (colony forming unit) analysis was done to distinguish BFU-E (burst-forming units-erythroid); CFU-GM (colony-forming unit-granulocyte, macrophage), and CFU-GEMM (colony-forming unit-granulocyte, erythroid, macrophage, megakaryocyte). Other stem cell types expressing $SH2^+$ were also analyzed. The data obtained via these studies are summarized below.

First, MNCs were harvested from placentas maintained for either 2 to 12 hours or for 12 to 24 hours. More MNCs were harvested from the placentas that had been maintained for 12 to 24 hours than from the placentas maintained for 2 to 12 hours. *See* Figure 1, Exhibit C-1.

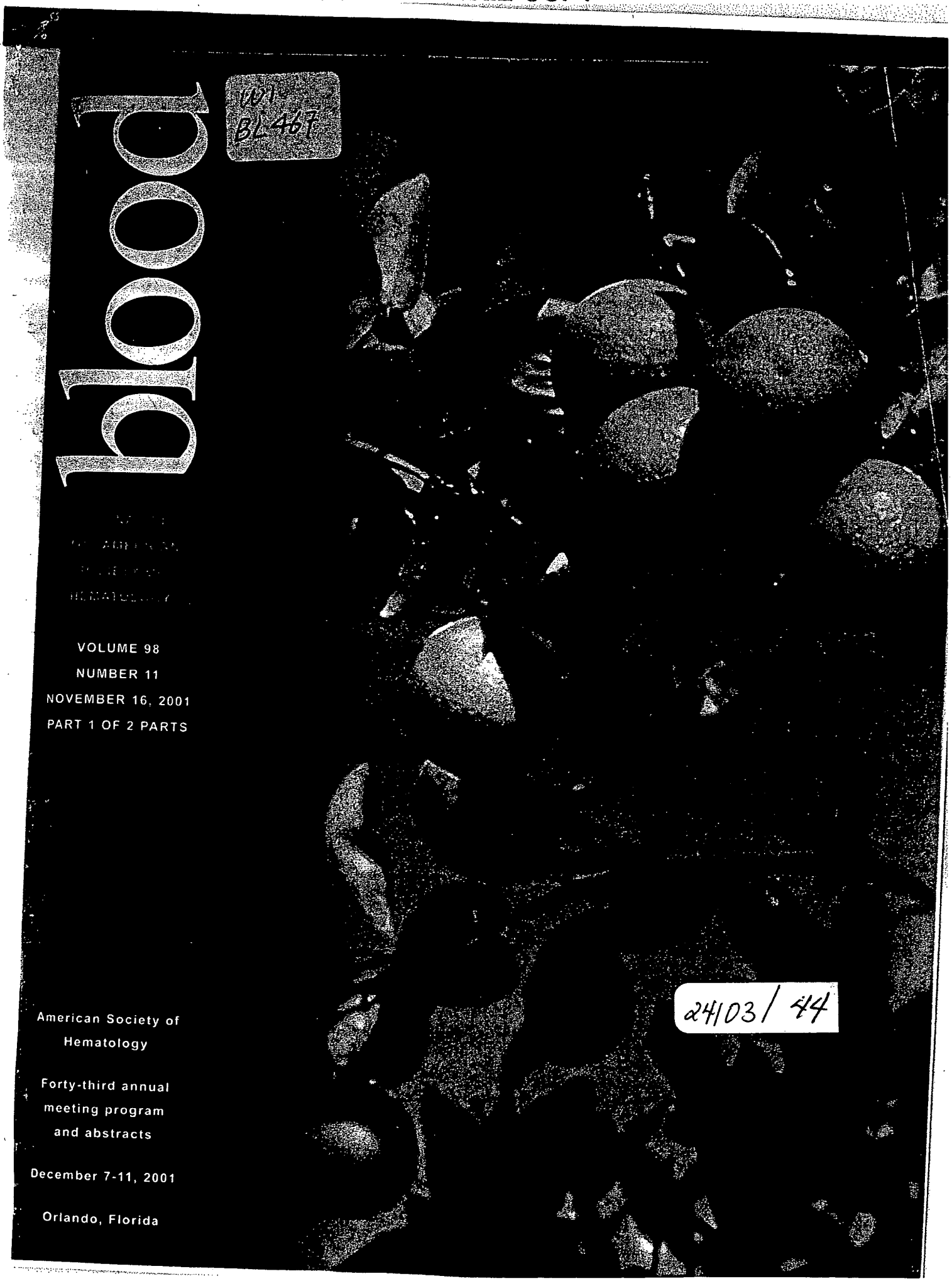
Figure 2 (Exhibit C-2) shows two examples of FACS analysis of $CD34^+CD38^-$ cells in placenta perfusate compared to two cord blood units. Table 1 (Exhibit C-2) shows quantitation of the $CD34^+CD38^-$ cells in the placenta perfusate compared to cord blood units. These data demonstrate that not only does placenta perfusate MNC contains stem cells, but it contains a higher proportion and total number of primitive stem cells ($CD34^+CD38^-$ cells) than that of cord blood. It is noted that it is thought that the $CD34^+CD38^-$ cells contain the long-term marrow repopulating activity.

Figure 3 (Exhibit C-2) shows three examples of CFU analyses of placenta perfusate MNCs. The placenta perfusates exhibited: CFU-E/BFU-E cells (66+/-11(%) of the total CFU population), which represent stem cells that give rise to erythrocytes; CFU-GM cells (28+/-10 (%) of the total CFU population), which represent stem cells that give rise to granulocyte and monocyte cells; and CFU-GEMM (6+/-3(%) of the total CFU population), which represent more primitive cells that can give rise to granulocytes, erythrocytes, monocytes, and megakaryocytes.

The studies also demonstrate that there are $SH2^+CD45^-$ and $SH3^+CD45^-$ type stem cells present in the placenta perfusate. (*See* Exhibit C-1; Fig. 4, Exhibit C-2.) Cells of these biomarker designations are indicative of non-hematopoietic stem cells. The data also reveal a larger amount of these cells in placenta perfusate compared to that from cord blood.

blood

VOLUME 98
NUMBER 11
NOVEMBER 16, 2001
PART 1 OF 2 PARTS



American Society of Hematology

Forty-third annual meeting program and abstracts

December 7-11, 2001

Orlando, Florida

Table 1 AUTOLOGOUS	Viability	TNC(%)	CD34+ (%)	CFC(%)	LTC-IC (%)
1(media)					
24					
48	94.6±3.6***	89.2±4.1***	83.0±13.3***	121.6±33.3*	102.2±18.5*
72	62.3±3.9***	58.2±1.4***	58.5±12.4***	70.0±18.9***	11.2±2.7***
Table 2 ALLOGENEIC					
24	87.5±2.0***	93.4±4.4*	102.8±14.9*	175.1±64.1*	155.8±31.7*
48	83.6±3.8***	82.1±3.5***	89.1±13.0*	180.0±54.9*	160.6±29.0***
72	72.6±3.7***	75.8±4.1***	77.1±12.0*	128.6±51.9*	165.7±21.0***

Results are expressed as mean±SEM; *p<0.05; n=15; n=5; n=7
% of control

Abstract# 768 **Poster Board #-Session: 768-I**

High Efficiency Recovery of Hematopoietic Progenitor Cells with Extensive Proliferative and Ex-Vivo Expansion Activity and of Hematopoietic Stem Cells with NOD/SCID Mouse Repopulating Ability from Human Cord Blood Stored Frozen for 15 Years. Hal E. Broxmeyer,[1,2,4] Edward F. Srour,[1,2,3] Giao Hangoc*,[1,2,4] Scott Cooper*,[1,2,4] Stacey A. Anderson*,[5] David Bodine.[5] *[1]Micro/Immuno and the Walther Onc Cntr, IU Sch of Med, Indpls, IN, USA; [2]Medicine, IU Sch of Med, Indpls, IN, USA; [3]Pediatrics and the Wells Cntr for Ped Res, IU Sch of Med, Indpls, IN, USA; [4]Walther Cncr Inst, Indpls, IN, USA; [5]GMBB, NHGRI, NIH, Bethesda, MD, USA.*

Cord Blood (CB) stem (HSC) and progenitor (HPC) cells have been used to treat malignant and non-malignant disorders. There have been over 2000 CB transplants performed with cells stored in CB Banks, however, the longest that a cord blood collection has been stored frozen prior to use for clinical transplantation is 3-5 years. Information on effects of longer term storage is important for CB banking. As part of our past efforts to evaluate feasibility of cryopreserving and using CB for transplantation (Broxmeyer et al 1989. PNAS 86:3828) we maintained stored frozen CB samples with pre-freeze information. This allowed us to quantitate efficiency of recovery of viable HPC from CB stored frozen 15 years ago. We were also able to evaluate the proliferative capacity of individual HPC, replating efficiency into 2° cultures of CFU-GEMM colonies (which offers an estimate of "self-renewal"), ex-vivo expansion capability of HPC, and in vivo mouse NOD-SCID repopulating capacity of HSC. The results were: The average recoveries (mean ± 1SD;N=9) of nucleated cells, CFU-GM, BFU-E, and CFU-GEMM for 15 year defrosts were respectively 83±12, 95±16, 84±25 and 85±25 with respective ranges of 64-100, 55-100, 29-100, and 29-100 using the same culture conditions as for the pre-freeze samples. Proliferative capacity of these cells in the presence of Epo, GM-CSF, IL-3 and steel factor was extensive, with colonies from CFU-GM, BFU-E and CFU-GEMM respectively containing 12,100±5,400 (2,500-22,500), 39,000±29,00 (10,000-182,500), and 198,100±52,900 (70,000-292,500) cells. Replating capacity of single CFU-GEMM colonies into 2° dishes which yielded any one type of progenitor colony, a CFU-GEMM colony, or colonies of CFU-GM, BFU-E plus CFU-GEMM, were repectively 98.6, 37.9, and 37.9% in the presence of the above cytokines in both 1° and 2° dishes. Defrosted cells were flow sorted into $CD34^+$, $CD34^+CD38^-$, and $CD34^+CD38^+$ populations and cultured in the presence of steel factor, Flt3-L, MGDF, IL-3, IL-6 and GM-CSF, with supplementation of cytokines every 48 hrs and demi-depopulation of culture every 7 to 10 days to assess ex-vivo expansion. $CD34^+$ cells isolated after thawing resulted in 8,000 to 19,000 fold expansion of nucleated cells from two samples respectively on days 57 and 41 of culture, with freshly isolated $CD34^+$ cells expanded 20,000 fold by day 70. HPC were expanded 26 and 17 fold from defrosted and 23 fold from fresh CB $CD34^+$ cells. In excess of 150 and 250 fold expansion of HPC were documented for $CD34^+CD38^-$ cells from the 15 year defrosts. Samples from five different defrosts were bead-separated into $CD34^+$ cells. Each sample was infused into two sublethally irradiated NOD/SCID mice, which were evaluated for human cell engraftment after 6-8 wks. All five samples demonstrated engraftment of $CD45^+$ human cells with multilineage phenotypes. These results demonstrate that human CB HSC and HPC cells with high proliferative, ex-vivo expansion and mouse NOD/SCID engrafting ability can be stored frozen for over 15 years and retrieved in viable form.

Abstract# 769 **Poster Board #-Session: 769-I**

Enhanced Recovery of Hematopoietic Progenitor and Stem Cells from Cultivated, Postpartum Human Placenta. Yalin Wang*, Qian Ye*, Joseph Cioffi*, Manoochehr Khorshidi*, Jory Magidson, Robert Katz*, Sarah MacIsaac*, Robert Hariri*. *Anthrogenesis Corporation, Cedar Knolls, NJ, USA.*

Hematopoietic progenitor and stem cells (HPSC) derived from umbilical cord blood (UCB) have many advantages over conventional sources of human graft material including superior proliferative capacity and lower risk of graft-versus-host disease. However, a persistent shortcoming has been the difficulty in recovery of sufficient volumes necessary to meet the cell dose requirements of adult recipients. We have developed a method to recover significant number of mononucleated cells (MNCs) and HPSCs from postpartum human placenta. Human placentas, procured under full informed consent of the donor, were perfused with 100 ml of heparinized, physiologic nutrient medium through umbilical artery and veins to remove residual blood after birth. The placentas were then cultivated for periods between 12 to 24 hours at 25oC and followed by a continuous perfusion at a controlled rate and pressure until a total of 200 to 250 ml of placenta perfusate (PLP) was collected. MNCs were isolated from PLP by differential centrifugation and density gradient fractionation. Populations of HPSC, characterized as $CD34^+$ cells including $CD34^+CD38^+$ or $CD34^+CD38^-$ were then quantified by flow cytometry (Table). The results showed that the total number of mononucleated and $CD34^+$ cells recovered from the cultivated, postpartum placenta was comparable to those recovered from a typical unit of cord blood collected by standard methods. More importantly, PLP contained a significantly higher ($p=0.01$) proportion of $CD34^+CD38^-$ cells,a cell subpopulation believed to contain the long term repopulating activity. The colony-forming unit (CFU) assays in methylcellulose clonal cultures demonstrated that MNCs from PLP contained 80+/-38 x10e3 (range: 47 to 150 x10e3) of total CFU including CFU-GM (28+/-10%), BFU-E/CFU-E (66+/-11%) and CFU-GEMM (6+/-3%). In addition, we detected a significant population of cells expressing mesenchymal stem cell markers SH2 and SH3, but not the hematopoietic marker CD45 (3.90+/-0.99% of $SH2^+CD45^-$ and 4.20+/-1.30% of $SH3^+CD45^-$) among MNCs from PLP. These cells were not detected in UCB. In conclusion, this method for procuring additional HPSC from postpartum placenta may be used to supplement UCB to yield sufficient graft material for transplantation in adults. The possible presence of mesenchymal stem cells in PLP may further improve the efficacy of HPSC engraftment.

Recovered From	Total MNC (x10e6)	CD34+ (%)	CD34+CD38- (%)	CD34+CD38+ (%)	Total CD34+ (x10e6)	Total CD34+CD38- (x10e6)
Cultivated Placenta (n=11)	788±658	0.38±0.18	0.12±0.1	0.26±0.18	0.77±0.22	0.28±0.2
Cord Blood (n=8)	619±270	0.72±0.56	0.05±0.03	0.67±0.56	1.05±0.75	0.07±0.06

Abstract# 770 **Poster Board #-Session: 770-I**

Immunological Properties of Allogeneic Blood Stem Cell Collections as Predictor of Outcomes after Transplantation. Shantaram S. Joshi,[1] Michael R. Bishop,[2] James C. Lynch*,[1] Iskra Pusic*,[1] John G. Sharp,[1] Stefano R. Tarantolo,[1] Victoria Whalen*,[1] Anne Kessinger,[1] Steven Z. Pavletic.[1] *[1]University of Nebraska Medical Center, Omaha, NE, USA; [2]National Cancer Institute, Bethesda, MD, USA.*

Some studies have suggested that alterations in numbers or function of cells mobilized for allogeneic blood stem cell transplantation (alloBSCT) may affect clinical outcomes posttransplant. The objective of this study was to test this hypothesis in a large group of patients. Mononuclear cells (MNC) were obtained from 98 G-CSF (5 or 10 µg/kg) mobilized HLA-identical related donor harvests and analyzed for immunophenotype and immunological function. These results were correlated in transplant recipients with major clinical endpoints including: rates of neutrophil or lymphocyte engraftment, acute or chronic graft-versus host disease (GVHD), relapse, and overall survival. Other analyses included influence on the recovery of lymphocyte subsets and immunological function at 100 days and one year posttransplant. All patients received transplants for a hematological malignancy and 91% were treated with a TBI-containing preparative regimen; GVHD prophylaxis was the same for all patients and consisted of cyclosporine and methotrexate. Graft cells were evaluated for immunophenotype, NK and LAK cytotoxicity against K562 and Raji cell lines, as well as for T-cell (PHA) and B-cell (LPS) mitogen function. Median numbers of cells infused $x10^6$/kg recipient weight were: CD3 - 5.8, CD4 - 3.8, CD8 - 1.8, CD19 - 1.2, CD3/CD25 - 0.33, CD56 - 0.47 and CD34 - 0.078. Median NK and LAK cytotoxicity at 50:1 effector: target ratios were 5.7% and 16.9% respectively. Median cpm responses to PHA (2.5 µg/ml) and LPS (200 µg/ml) were 33,515 and 5,856 respectively. The only statistically significant associations with clinical outcomes were the following: Infusions of CD34 cells above the median value were connected with faster posttransplant recovery to 500 lymphocytes (RR 1.6, P value 0.029). Better responses to the B-cell mitogen LPS were associated with higher risk of acute GVHD grades II-IV (RR 2.8, P value <0.01). These data suggest that standard immunological assays performed on T-cell repleted alloBSCT grafts do not provide adequate information on clinical outcomes. This should be considered when planning allograft engineering trials.

Abstract# 771 **Poster Board #-Session: 771-I**

Long-Term Follow-Up and Safety of Normal Peripheral Blood Progenitor Cell (PBPC) Donors Treated with Filgrastim: The MD Anderson Cancer Center Experience. Paolo Anderlini,[1] Martin Körbling,[1] Ka-Wah Chan,[1] Richard Champlin,[1] Sara Strom*.[2] *[1]Department of Blood & Marrow Transplantation and Epidemiology, The University of Texas MD Anderson Cancer Center, Houston, TX, USA; [2]Department of Epidemiology, The University of Texas MD Anderson Cancer Center, Houston, TX, USA.*

Background: Data on long-term safety of filgrastim administration in normal PBPC donors are scarce. In the view of the known biologic activity of filgrastim, the main theoretical risk is believed to be the possible, late development of acute or chronic leukemia. **Material and Methods:** We conducted a survey of the PBPC donors enrolled and registered in the PBPC collection protocol at our institution between 1994 and 1998. This time period was selected to ensure adequate long-term follow-up. The study was approved by the Institutional Review Board and the participants provided informed consent. A total of 396 PBPC donors were included in the database. They underwent filgrastim mobilization (for 3-5 days on average) and PBPC apheresis. The donors were interviewed by telephone between December 1998 and February 2000. Fifty-three donors were excluded from the survey (forty-nine were not living in the US, three had blank records/no donor name available, and one person did not actually donate). **Results:** Of the remaining 343 donors, 281 (82%) were interviewed. The reasons for the lack of interview (n=62) were: no current contact information available n=36 (10%), declined n=10 (3%), no response despite multiple contacts n=14 (4%), and two had died (one suicide, one grand mal seizure, both seemingly unrelated to the donation). The mean age at donation was 44 years (range 5-77), 47% male and 53 % female. Seventy-six percent were Caucasian, 17% were Hispanic, 5% were African-American, and 1% other ethnicities. The donor was usually the sibling of the recipient (96%), with 4% being some other blood relative. The median follow-up after PBPC donation was 39 months (range 7-80), 99% had at least one year of follow-up. Six donors had donated stem cells at other institutions. At the time of the interview none of the donors had been diagnosed with acute or chronic leukemia. **Conclusion:** Although the sample size is small and the follow-up is limited, these data do suggest that brief (3-5 days) exposure to filgrastim during PBPC donation is not associated with any obvious or striking long-term (3-4 years) risk for the development of leukemia.